Taxonomic rank of *Tanacetum boreale* Fisch. ex DC. (Asteraceae)

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**ABSTRACT:** This article presents the results of population-genetic analysis in four populations of *Tanacetum vulgare* (typical tansy) from Southern Siberia on the basis of the Randomly Amplified DNA Fingerprints (RAF). We used RAF to find certain molecular-genetic differences between the typical tansy and the northern tansy. Since the present results did not show any clear differences between the typical tansy and the northern tansy at species level, we suggest that *T. vulgare* includes two subspecies: the typical *T. vulgare* subsp. *vulgare* and *T. vulgare* subsp. *boreale*.

**KEYWORDS:** Asteraceae, DNA, Multidimensional scaling, Populations, Population genetics, RAF, Southern Siberia, *Tanacetum*, tansy, taxonomy, UPGMA

*Tanacetum vulgare* L. (tansy, Asteraceae) is an official plant with a great value. Drugs from inflorescences of tansy have choleretic, anti-inflammatory, antimicrobial, antihelminthic and insecticidal actions; influence as antipyretic, antispasmodic, vasodilator medicines (Kulikov 1973; Vydrina and Shreter 1980; Safonov 2005).

The typical tansy grows in the northern hemisphere, has basically Euro-Asiatic type of area (Tzvelev 1961; Vydrina and Shreter 1980; Wagenitz 1992) (Fig. 1). *Tanacetum vulgare* is distributed in European part of USSR, except eastern regions of Ciscaucasia, Transcaucasia, and lower reach of Volga and Ural Rivers; in Eastern Siberia, Far East, Eastern Kazakhstan and Kyrgyzstan only as adventitious (Vydrina and Shreter 1980). As adventitious plant it grows in some regions of Middle Asia, while that can be found in North America where introduced from other countries (Tzvelev 1961; Wagenitz, 1992).

In “Flora of Siberia” (Boldyreva 1997) *T. vulgare* was subdivided into two subspecies such as the typical subspecies and *T. vulgare* subsp. *boreale*. G. Wagenitz (1992) considered northern tansy as *T. vulgare* subsp. *boreale* also.


Some authors consider the northern tansy as separate species *T. boreale* (Tzvelev 1961; Vydrina and Shreter 1980). As Tzvelev (1961) recorded, *T. boreale* and *T. vulgare* had different diameter of wrapper: *T. boreale*: 7–13 mm, and *T. vulgare*: 5–8 mm. Tzvelev (1961) believed, that *T. boreale* was linked with typical species by multiple transitional samples and differed from it not so much leaf dissection, as larger and relatively not numerous baskets with wider dark brown membranous edge. He noted, there were no clear samples of *T. boreale* from European part of USSR, and existing records were *T. vulgare* with more dissected leaves.

*Tanacetum boreale* was described on the basis of garden samples, originated, probably, from the Far East. The holotype of the species is located in Berlin, Germany; the isotype of the species could be situated in Saint Petersburg, Russia (Tzvelev 1961).

In the taxonomic treatment of *Tanacetum* for the territory of the Soviet Far East (Barkalov 1992) the authors took the view about species rank of *T. boreale* also. They noted that *T. boreale* varied in basket size and color of the edge of wrapper leaflets. On south of Amur region and Primorye the specific form of *T. boreale* grows, which has multiple small baskets and wrapper leaflets often with light-brown edge (Barkalov 1992).

Thereby, there is no unified opinion about the composition of *T. vulgare*. Meanwhile, the species is a medicine plant and is included in the official pharmacopoeia of various countries, for example, Russian Federation, Belgium, Portugal, Finland (Tyulin and Bogatkins 1993), so it is necessary to establish clear boundaries between these 2 taxa of *Tanacetum* for its future use.

Thus, these two subspecies of *T. vulgare* can be very interesting to find differences in molecular systematics. So
the purpose of our study is to clear, is it reasonable to
distinguish T. vulgare subsp. boreale in a separate species,
and its isolation degree from the typical subspecies. The
objective to reach this purpose was to know, whether these
taxa growing in Southern Siberia have been genetically
isolated or they have been cross-hybridized to exchange
certain genetic materials.

For solution of the objective to absorb this
taxonomical problem we have carried out
population-genetic analysis using the randomly amplified
DNA fingerprints (RAF). This method is a slightly
modified method of DNA amplification fingerprinting
(DAF) (Waldron et al. 2002) and sensitive enough to
divide closely related forms. The high degree of
polymorphism of amplified fragments in RAF allows to
clear the genetic differences between separate specimens,
that might be used in determination of species independence if disputable issue. This method is optimal
to detect the genetic diversity of plants with previously
unexplored genome (Waldron et al. 2002).

MATERIALS AND METHODS
The plant materials were collected in four locations of the
areas where the two tansy subspecies were grown
sympatrically – in the territory of Southern Siberia (Fig. 1).
Plant materials such as leaves were collected in August
2010: ten samples of T. vulgare subsp. vulgare
were collected in Altai krai, Alei region, 10 km eastward vil. Uzhum; Kemerovo Region, M53 Highway between the vil. Verkh-Chebula and vil. Petropavlovka; ten samples in
Khakass Republic, vil. Izyhskie kopi, bank of River
Abakan; nine samples of T. vulgare subsp. boreale –
Krasnoyarsk krai, Oi pass. These materials were dried
with silica gel.

DNA isolation DNA was extracted by using Diamond
DNA kit (ABT Llc., Russia) in accordance with
instructions of the manufacturer.

RAF The amplifications were carried out to select the
primer. For primer selection we used primers of series A:
01-10 and those of series RAF: K-01 5’-CATT CGAGCC
-3′, K-01a 5’-CATT CGAGCA-3′, K-01b 5’-CATT CG
AGCG-3′, K-02 5’-GTCTCCGCA-3′, K-02a 5’-GTCT
CCGCAC-3′, K-02b 5’-GTCTCCGCAG-3′ (Waldron et
al. 2002).

For the further analysis we chose the primer RAF
K-02a, which gives polymorphic amplification product.
Polymerase chain reactions (PCR) were carried out in
25 microliter of reaction mixture, consisting of 2
microliter of DNA sample, 2.5 microliter of 10X buffer,
and 25 mM MgCl2, 2 microliter of 10 μM primer, 1
microliter of a mixture of 5 nM dNTPs, on a thermo-
cycler MyCycler (Bio-Rad, USA), using the program
RAF: 94.0 °C for 5 min. [94.0 °C for 30 sec., 57.0 °C for 1
min., 56.0 °C for 1 min., 55.0 °C for 1 min., 54.0 °C for 1
min., 53.0 °C for 1 min.] x35, 72.0 °C 10 min., 4.0 °C –
storage.

Analysis of amplification products DNA fragments
were separated by electrophoresis using an automated
electrophoresis station Experion (Bio-Rad, USA) with a
reagent kit for microfluidic analysis on chips Experion
DNA 1K Analysis Kit (Bio-Rad, USA). The analysis
results are presented as a virtual gel in Fig. 2.

Statistical processing of the results The phenetnic
analysis matrix was made in Microsoft Excel based on the
presence (1) or absence (0) of fragments of the same
length as determined by applying a line for each sample in
the photograph of the gel electrophoresis. Further analysis
used 47 fragments for 39 samples. The matrix was
analyzed in program for phonetic analysis NTSYS-pc,
Pairwise genetic distances were calculated using the
Jaccard coefficient (Jaccard 1908), based on which the
data were processed by UPGMA (unweighted pair group
method with arithmetic mean) method. Multidimensional
scaling (Kruskal 1964) was also carried out in the program NTSYS-pc.

RESULTS
In recent years RAF has been sufficiently applied to value the genetic diversity and interpopulational relations in plants (Cunningham et al. 2002; Ramage et al. 2004; Nand et al. 2005; Chan et al. 2008; Kutsev et al. 2013a, b; Kreshchenok et al. 2016). For example, RAF supported to confirm the division of *Cassia brewsteri* (F.Muell.) F.Muell. ex Benth in two subspecies, which could cross in overlapping zone of their area of distribution (Cunningham et al. 2002). We have used this method at earlier period to study the genetic diversity of some *Sanguisorba* L. Fifty-two characters (fragments) samples were divided into two clusters: the first cluster consisted of samples of *S. alpina* Bunge and *S. azovtsevii* Krasnob. et Pschen., while the second cluster consisted of samples of *S. officinalis* L. These results are in conflict with the hypothesis of hybrid origin of *S. azovtsevii* and shows allopolyploid origin of this species on the basis of *S. alpina* with small part of *S. officinalis* (Kutsev et al. 2013a). Additionally this method was used for genotyping varieties of common and durum wheat (Kutsev et al. 2013b). In this study RAF showed two separate clusters of common wheat varieties and durum wheat varieties with little overlap.

In the present study of *Tanacetum vulgare* the polymorphism of amplified fragments, identified using cluster analysis and multidimensional scaling, showed no significant differences between samples of *Tanacetum vulgare* subsp. *boreale* and *T. vulgare* subsp. *vulgare* (Fig. 3).

Results of the analysis indicate a lack of differentiation of subspecies within southern Siberia. It means that these taxa are not genetically isolated from each other in Southern Siberia and they could hybridize the genetic materials.

Insignificant differences might indicate that there are transitional forms on the territory of collecting specimens. Probably, in the further work the material should be collected and studied from the parts of area not overlapping.

DISCUSSION
The northern tansy gives place to the typical tansy in Far East, Arctic, Eastern Siberia, in north and mountain regions of Western Siberia, Eastern Kazakhstan and Kyrgyzstan, where grows up from the lowlands to the highlands. The northern border of its area reaches 70°N, near Norilsk, Khatanga, in lower Lena and Kolyma Rivers and near Anadyr Mouth. The northern tansy occurs in Kamchatka, Okhotsk seaside, Sakhalin Island, in Primurye and Primorye, Transbaikal, Dzungarian Alatau, and Central Tien Shan (Vydrina and Shreter 1980). *Tanacetum vulgare* subsp. *boreale* can be found in Hokkaido (Japan) – in Souya, Kushiro, Abashiri, along the River Tokachi, and so on, as native plant.

The borders between areas of distribution of these two taxa are fuzzy, and there are transitional forms in the overlapping zone (Fig. 1).

The subspecies grow under the similar ecological conditions. The typical tansy grows on meadows, margins, in steppes, by riversides, in sparse mixed and birch forest,
Northern tansy differs from the typical one with respect to more expressed downiness, larger and less numerous baskets with dark brown broad membranous edge on wrapper leaflets and more dissected leaves (Table 1).

Thereby, there are no significant differences between Tanacetum vulgare subsp. vulgare and T. vulgare subsp. boreale at species level. They have many transitional forms in overlapping region of the areas of their distribution. We believe there is no reason to give northern tansy the species rank. Taking into account the complex of

Table 1. Morphological comparison of the typical tansy and the northern tansy.

<table>
<thead>
<tr>
<th>Character</th>
<th>Tanacetum vulgare subsp. vulgare (Common tansy)</th>
<th>T. vulgare subsp. boreale (Northern tansy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wrapper diameter</td>
<td>5–8 mm</td>
<td>7–13 mm</td>
</tr>
<tr>
<td>Stem leaves</td>
<td>Terminal lobes up to 5 mm width,</td>
<td>Terminal lobes of pinnately</td>
</tr>
<tr>
<td></td>
<td>toothed by edge or smooth-edged,</td>
<td>decompound 1st order segments</td>
</tr>
<tr>
<td></td>
<td>shortly peaked on top</td>
<td>usually more straddling and narrower</td>
</tr>
<tr>
<td>Number of baskets</td>
<td>Up to 120</td>
<td>Up to 15</td>
</tr>
<tr>
<td>Plant height</td>
<td>Up to 150 cm</td>
<td>Up to 70 cm</td>
</tr>
<tr>
<td>Edge of wrapper leaflets</td>
<td>Narrow, light</td>
<td>Wide, dark</td>
</tr>
</tbody>
</table>
morphological and molecular-genetic characters we have concluded northern tansy as *Tanacetum vulgare* subsp. *boreale*.

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**LITERATURE CITED**


